## REMARKS

I. Interview

Applicants thank Examiner Belyavskyi and Examiner Christina Chan for the courtesies extended in a telephonic interview on October 8, 2002, during which the pending rejections and restriction requirement were discussed. The undersigned and Dr. Mario Aussemacher participated on behalf of the Applicants.

Claims 14-21, 33, and 88-97 are under examination.

II. Rejections Under 35 U.S.C. § 102

a) Rejection over Manz et al., PNAS 1995 92:1921-25

Claims 14-17, 33, 88-92, 94 and 97 were rejected as allegedly anticipated by Manz et al. Applicants respectfully traverse this rejection.

As discussed during the October 8, 2002 interview, to anticipate, a prior art reference must disclose each element of the claimed invention. The invention includes the step of antigen-specific stimulation of T-cells. See, e.g., claims 14 and 88, and the specification at, e.g., page 23, lines 12-17. The Manz et al. reference did not describe antigen-specific stimulation of T-cells, and instead discloses that murine splenic lymphocytes were activated *in vitro* with staphylococcal enterotoxin B, a "superantigen." See Manz et al. at page 1924. Stimulation of T cells by a superantigen is not antigen-specific stimulation. Superantigens act by binding a MHC class II molecule and the $V_\beta$ region of the T-cell receptor, causing activation of large numbers of T cells in a population and resulting in massive production of cytokines by $CD_4$ T cells. Characteristics of superantigens are well known in the art and are described in, for example, IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE, 2nd edition, by Charles A. Janeway, Garland Publishing Inc, New York at 4:24 and 4:38. These pages were made of record in Applicant's response filed February 16, 2001. At the request of the Examiner, a new copy is enclosed. The attention of the Office is also respectfully directed to paper no. 10 and the amendment filed February 16, 2001, illustrating that the issues raised by the Office are old, having already been discussed (and overcome) at the earliest stages of the instant prosecution.

Because Manz et al. does not teach each element of the claimed invention it cannot anticipate the claims. Applicants respectfully request withdrawal of the § 102(b) rejection of claims in view of Manz et al.

b) Rejection over Assenmacher et al., Eur. J. Imm. 1998 28:1534

Applicants respectfully traverse this rejection of claims. Assenmacher et al. describe the kinetics of secretion of cytokines by enterotoxin B-activated T helper lymphocytes (see title). As noted above, staphylococcal enterotoxin B is a superantigen, not an antigen specifically recognized by a T cell receptor. As noted above, enterotoxin B does not cause antigen-specific stimulation of T-cells, as recited in the claims. Thus, Assenmacher et al. could not anticipate the present invention.

In addition, it is believed that the Assenmacher et al. reference is not prior art to the claimed invention. The present application is entitled to the priority date of provisional patent application 60/085,136 (filed May 11, 1998). See § III, *infra*. According to Applicants' inquiries to the publisher, it is believed the May 1998 issue of the European Journal of Immunology (Volume 28, No. 5) had a nominal publication date of May 5, 1998. However, inquiry to libraries at four U.S. universities, and 15 European universities, indicated that subscribers did not receive the journal until after May 11, 1998. Becaue it was not publicly available until after the application filing date, it is not available as prior art.

For both of these reasons, the Assenmacher et al. does not anticipate the present invention. The Examiner is requested to consider each reason independently, as each is sufficient to establish the absence of anticipation.

c) Rejection over Miltenyi et al. (WO 94/09117)

Claims 1-4 and 8-50 stand rejected under § 102(b) as allegedly anticipated by Miltenyi et al. (WO 94/09117). Applicants traverse this rejection of claims.

WO 94/09117 describes a method for labeling cells with products they secrete. WO 94/09117 does not describe the step of exposing T cells to an antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen-specific T cell, as is recited in the present claims. Because Miltenyi et al. does not teach each element of the claimed invention it cannot anticipate the claims. Applicants respectfully request withdrawal of the § 102(b) rejection of claims in view of Miltenyi et al.

III. Entitlement to Benefit of Priority Application 60/085136 (Paragraph 2 of the Office Action)

The Office has asserted that the present claims are not entitled to benefit of the filing date of the priority application, provisional application 60/085136, because allegedly the provisional application:

> "does not support the claimed limitations of the instant application, encompassing methods of label[ing] antigen-specific T cells, wherein the secreted product specifically bound to the capture moiety."

Applicants respectfully maintain that support for the pending claims is replete in the provisional application. For example, compare claim 14 as found in the priority application with claim 14 as now pending. Differences in the claim filed in the provisional application and claim 14 as now pending in the instant application are noted by underscoring.

| 60/085,136 | 09/309,199 |
|---|---|
| A method labeling antigen-specific T cells with a product secreted and released by the cells, wherein the product is secreted in response to antigen stimulation, which method comprises: | A method to label antigen-specific T cells with a product secreted and released by the cells, wherein the product is secreted in response to antigen stimulation of said T cells, which method comprises: |
| exposing the cells to at least one antigen under conditions effective to elicit antigen-specific stimulation of at least one T cell; and | a) exposing the antigen-specific T cells to at least one antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen specific T cell; |
| modifying the surface of the cells to contain a capture moiety specific for the product; | b) modifying the surface of the antigen-specific T cells to contain a capture moiety specific for the product; and |
| culturing the cells under conditions wherein the product is secreted, released and specifically bound to the capture moiety, thereby labeling the product-secreting cells. | c) culturing the cells under conditions wherein the product is secreted, released and specifically bound to the capture moiety, thereby labeling the product-secreting cells, wherein steps a), b), and c) can be performed in any order or simultaneously. |

In addition to showing the extensive similarity between the claims of the priority application and the claims under examination, this chart illustrates support in the priority application of both elements alleged by the Office not to be present in the priority application:

- A method of labeling antigen-specific T cells
- wherein the secreted product is specifically bound to the capture moiety.

Additional support is found throughout the provisional specification. See, for example, the section of the specification captioned "Cell labeling" at page 24, line 24 to page 29, line 23. Also see, e.g., page 9, line 25 to page 10, line 2, and page 12, lines 7-10 of the specification. Further, it will be apparent that methods of cell "isolation" and "analysis" described extensively in the specification entail "labeling" cells.

For clarity of the prosecution history, the Examiner is respectfully requested to acknowledge that the pending claims find support in the priority application.

IV. Other Matters

a) Formal Drawings are submitted herewith (paragraph 3 of the Office Action).

b) Applicants no longer claim small entity status. Accordingly, at the time of payment of the Issue Fee in the instant application, Applicants will submit payment as a large entity.

## CONCLUSION

Applicants respectfully submit that all issues that were raised in the outstanding Office Action have been addressed. Accordingly, reconsideration and allowance of the pending claims are respectfully requested. If it is determined that a telephone conversation would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 212302000720.

Respectfully submitted,

Dated: November 12, 2002 By: _____

Randolph Ted Apple
Registration No. 36,429

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5933
Facsimile: (650) 494-0792

# IMMUNOBIOLOGY

## THE IMMUNE SYSTEM IN HEALTH AND DISEASE

## SECOND EDITION

**Charles A. Janeway, Jr.**
Yale University Medical School

**Paul Travers**
Birkbeck College, London University

CB CURRENT BIOLOGY LIMITED
Current Biology Ltd
London, San Francisco and Philadelphia

CHURCHILL LIVINGSTONE
Churchill Livingstone
Edinburgh, London and New York

GP
Garland Publishing Inc
New York and London

**Text editors:** Miranda Robertson. Eleanor Lawrence
**Project editors:** Emma Hunt, Emma Dorey
**Illustrator:** Matthew McClements
**Layout:** Huw Woodman
**Production:** Kate Oldfield
**Software support:** Gary Brown
**Proofreader:** Melanie Paton
**Indexer:** Maija Hinkle



**Distributors**
*Inside North America*: Garland Publishing Inc., 717 Fifth Avenue, New York, NY 10022. USA.
*Inside Japan*: Nankodo Co. Ltd., 42-6, Hongo 3-Chome, Bunkyo-ku, Tokyo 113, Japan.
*Outside North America and Japan*: Churchill Livingstone, Robert Stevenson House, 1-3 Baxter's Place, Leith Walk, Edinburgh, EH1 3AF.

ISBN 0-443-05658-7 (paperback) Churchill Livingstone
ISBN 0-443-05659-5 (paperback) International Student Edition

A catalog record for this book is available from the British Library.

**Library of Congress Cataloging-in-Publication Data**
Janeway, Charles.
Immunobiology: the immune system in health and disease/
Charles A. Janeway, Jr., Paul Travers.—Second ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-8153-2044-2 (pbk.).
1. Immunity. 2. Immune System. I. Travers, Paul, 1956- .
II. Title
QR181.J37 1996
616. 07'9—dc20

95-49564
CIP

This book was produced using Corel Ventura Publisher 5.0 and CorelDRAW 5.0.

Printed in Singapore by Stamford Press.

Published by Current Biology Ltd., Middlesex House, 34-42 Cleveland Street, London W1P 5FB. UK and Garland Publishing Inc., 717 Fifth Avenue, New York, NY 10022. USA.

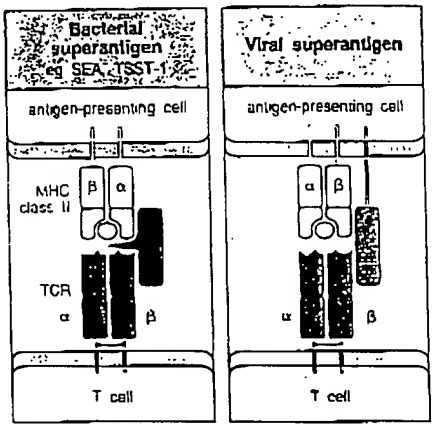
## 4-24 Diversity in T-cell receptors is not extended by somatic hypermutation.

When we discussed the generation of antibody diversity in Section 3-18, we saw that somatic hypermutation increases the diversity of all three complementarity determining regions of both immunoglobulin chains. As a general principle, somatic hypermutation does not occur in T-cell receptor genes, so that variability of the CDR1 and CDR2 regions is limited to that of the germline V gene segments. Somatic mutation has been reported for the α chain V regions expressed by T cells isolated from germinal centers, but it is not known whether these mutated α chains contribute to a cell-surface T-cell receptor and so generate an altered specificity. At present, therefore, it appears as though the bulk of diversity in T-cell receptors is generated during rearrangement and is consequently focused on the CDR3 regions.

Why T-cell and B-cell receptors differ in their ability to undergo somatic hypermutation is not clear, but several explanations can be suggested on the basis of the functional differences between T and B cells. Because the central role of T cells is to stimulate both humoral and cellular immune responses, it is crucially important that T cells do not react with self proteins. T cells that recognize self antigens are rigorously purged during development (see Chapter 6), and the absence of somatic hypermutation helps to ensure that somatic mutants recognizing self proteins do not arise later in the course of immune responses. This constraint does not apply with the same force to B-cell receptors, as B cells usually require T-cell help in order to secrete antibodies. A B cell whose receptor mutates to become self reactive would, under normal circumstances, fail to make antibody for lack of self-reactive T cells to provide this help. An additional argument might be that T cells already interact with a self component, namely the MHC molecule that makes up part of the ligand for the receptor, and thus might be unusually prone to developing self-recognition capability through somatic hypermutation. In this case, the obverse argument can also be made: because T-cell receptors must be able to recognize self MHC molecules as part of their ligand, it is important to avoid somatic mutation that might result in the loss of recognition and consequent loss of any ability to respond. However, the most likely explanation for this difference between immunoglobulins and T-cell receptors is the simple one that somatic hypermutation is an adaptive specialization for B cells alone, because they must make very high affinity antibodies in order to capture toxin molecules in the extracellular fluids. We shall see in Chapter 9 that they do this through somatic hypermutation followed by selection for antigen binding.

## 4-25 Many T cells respond to superantigens.

Not all antigens that bind to MHC class II molecules are presented as peptides in the peptide-binding groove. A few fall into a distinct class known as **superantigens**, which have a distinctive mode of binding that enables them to stimulate very large numbers of T cells, often with disastrous consequences. Superantigens are produced by many different pathogens, including bacteria, mycoplasmas, and viruses, and bind directly to MHC molecules without being previously processed; indeed, fragmentation of a superantigen destroys its biological activity. Instead of binding in the groove of the MHC molecule, superantigens bind to the upper surface of both the MHC class II molecule and the $V_\beta$ region of the T-cell receptor (Fig. 4.33). Thus, the α chain V region and the DJ junction of the β chain of the T-cell receptor have little effect on superantigen recognition, which is determined largely by the V gene segment



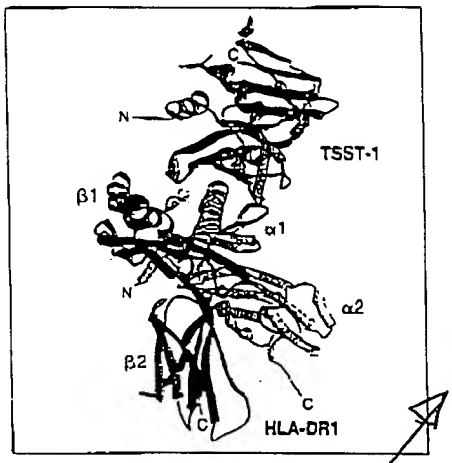




**Fig. 4.33 Superantigens bind directly to T-cell receptors (TCR) and to MHC molecules.** Superantigens interact with MHC class II molecules and T-cell receptors in a way that is quite distinct from the way that normal peptide antigens bind. Superantigens can bind independently to MHC class II molecules and to T-cell receptors, binding to the β chain of the T-cell receptor, away from the complementarity determining region, and to the outer faces of the MHC class II molecule, outside the peptide-binding site. Two distinct classes of superantigens have been described so far. Bacterial superantigens, like staphlyococcal enterotoxins (SEs) or the toxic shock syndrome toxin (TSST-1) are soluble proteins secreted by bacteria. The so-called endogenous viral superantigens are proteins expressed by some viruses that infect mammalian cells and integrate into the DNA of their host. The best characterized viral superantigens are membrane proteins produced by endogenous viruses of mice, especially the murine mammary tumor virus, MMTV. The crystal structures of complexes of MHC class II molecules with staphylococcal enterotoxin B (SEB) and with TSST-1 have been determined (that of the HLA-DR1:TSST-1 complex is shown in the bottom panel) and show clearly that the superantigens binds to the α chain of the class II molecule. In the case of the TSST-1 superantigen, peptides bound by the class II molecule and parts of the β chain may also contact the superantigen and influence its binding. The viral superantigen MMTV-7 binds to the β chain of MHC class II molecules. Each of these superantigens contacts a separate site on the T-cell receptor Vβ domain. Bottom panel courtesy of J Kim.

that encoded the β chain. Each superantigen can bind one or a few of the different products of Vβ gene segments, of which there are 20–50 in mice and humans, so a superantigen can stimulate 2–20% of all T cells.

This mode of stimulation is not specific for the pathogen and thus does not lead to adaptive immunity. Instead, it causes massive production of cytokines by CD4 T cells, the predominant responding population. These cytokines have two effects on the host, systemic toxicity and suppression of the adaptive immune response. Both of these effects contribute to microbial pathogenicity. Among the bacterial superantigens are the **staphylococcal enterotoxins** (SE) that cause common food poisoning and the **toxic shock syndrome toxin** (TSST).

The role of viral superantigens is less clear. Endogenous viral superantigens are very common in mice, and we shall see in Chapter 6 that the study of these superantigens has played a critical role in elucidating one of the major mechanisms of self tolerance.

## 4-26 The T-cell receptor associates with the invariant proteins of the CD3 complex.

Neither chain of the T-cell receptor heterodimer has a large cytoplasmic domain that might serve to signal the cell that the T-cell receptor has bound antigen. Instead, that function is carried out by a complex of proteins, known as the **CD3 complex**, which is stably associated with the T-cell receptor on the surface of T cells (Fig. 4.34). The complex consists of three distinct proteins with some homology to immunoglobulins, and two other proteins not homologous to immunoglobulins but closely related to each other. Unlike the T-cell receptor heterodimer, the CD3 proteins have cytoplasmic extensions that allow them to interact with signal-transducing proteins. The proteins showing sequence homologies to immunoglobulins are known as CD3γ, CD3δ, and CD3ε. These three proteins, like Igα and Igβ, consist of extracellular domains that bear weak amino acid homology to immunoglobulin domains, a transmembrane region, and modest cytoplasmic domains. The transmembrane